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The Effects of Acute Doses of Standardized *Ginkgo biloba* Extract on Memory and Psychomotor Performance in Volunteers

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This study investigated the effects of acute doses of *Ginkgo biloba* extract (GBE) on memory and psychomotor performance in a randomized, double-blind and placebo controlled 5-way cross-over design. Thirty-one volunteers aged 30–59 years received GBE 150 mg (50 mg t.d.s), GBE 300 mg (100 mg t.d.s.), GBE 120 mg *mane* and GBE 240 mg *mane* and placebo for 2 days. Following baseline measures, the medication was administered at 0900 h for the single doses and at 0900, 1500 and 2100 h for the multiple doses. The psychometric test battery was administered pre-dose (0830 h) and then at frequent intervals until 11 h post dose.

The results confirm that the effects of GBE extract on aspects of cognition in asymptomatic volunteers are more pronounced for memory, particularly working memory. They also show that these effects may be dose dependent though not in a linear dose related manner, and that GBE 120 mg produces the most evident effects of the doses examined. Additionally, the results suggest that the cognitive enhancing effects of GBE are more likely to be apparent in individuals aged 50–59 years.

Keywords: Ginkgo biloba extract; acute doses; asymptomatic volunteers; memory; cognitive; psychomotor performance.

INTRODUCTION

The dried green leaves of the *Ginkgo biloba* tree provide the crude drug from which standardized ginkgo extracts are obtained. The 1994 German Commission E monograph describes standardized extracts of *Ginkgo biloba* extract (GBE) as containing 22%–27% flavonoid glycosides and 5%–7% terpene lactones consisting of specified amounts of ginkgolides and bilobalides. Other chemicals present in the extract include a number of organic acids such as hydroxykynurenic acid, shikimic acid and vanillic acid (Schulz *et al.*, 1996).

GBE appears to have a litany of pharmacological activity manifesting itself in improvement in symptoms of circulatory diseases and degenerative cognitive diseases in the elderly (Sitzer, 1987ab Weitbrecht and Jansen, 1986). Although the exact role of GBE is not clearly understood, beneficial activity appears to be associated with the free radical scavenging activity of the flavone glycoside content and platelet activating factor (PAF) inhibition due to the ginkgolides constituents (Braquet *et al.*, 1985; Pincemail and Deby, 1988).

The principle therapeutic use of GBE in Europe is in the treatment of cerebral dysfunction or cerebral insufficiency. This condition manifests itself through cerebral ischaemia that is associated with hypoxia, hypoglycaemia and a reduced ability to remove waste products by the blood from the cerebral regions (De Feudis, 1998). Cerebral insufficiency is also associated with cognitive impairment, which can range from mild cognitive decline to the more severe types of senile dementias of primary degenerative (e.g. Alzheimer's disease), vascular and mixed origins. The more severe type of cognitive impairment associated with dementia involves a pattern of impairment in which memory, abstract thinking, attention, psychomotor functioning, mood, personality and social functioning are all simultaneously impaired (DSM IV, American Psychiatric Association, 1994).

Clinical studies have shown that GBE is efficacious in treating mild cerebral dysfunction as well as the more severe types of senile dementia. Weitbrecht and Jansen (1986) found significant improvements in patients suffering from slight to moderate primary degenerative dementia on psychometric tests and clinical rating scales after 4 weeks of treatment with GBE, while Sitzer (1987ab) found significant improvements in cerebral blood flow patterns, vigilance and normalization of EEG patterns in patients with symptoms of cerebrovascular insufficiency who were treated with GBE over 12 months. More recently, Le Bars *et al.* (1997) have shown that GBE improved the cognitive performance and social functioning of demented patients for 6 months to 1 year.

The beneficial effects of GBE in these studies could be related to several different types of action including an improvement in cerebral circulation and associated increases in oxygen and nutrient supply to the central nervous system. In addition, the free radical-scavenging activity of GBE could prevent excessive lipid peroxidation and cell damage and thus delay or prevent the symptoms of Alzheimer's disease and other neurodegenerative disorders. Heiss and Zeiler (1978) found that GBE increased global cerebral blood flow (CBF) by about

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8.4% and improved tissue perfusion in ischaemic areas in patients with cerebrovascular ischaemia while Tea *et al.* (1979) found significant increases in glucose and oxygen consumption in patients with neurologic ischaemic syndrome.

Direct effects of GBE on cognitive function in normal asymptomatic volunteers have been shown after the administration of both acute (Subhan and Hindmarch, 1984a; Allain *et al.*, 1993) and chronic doses (Warburton, 1986). The most consistent results have been for memory. Subhan and Hindmarch (1984a) showed that 600 mg GBE significantly improved short term (working) memory as assessed using the Sternberg technique (Sternberg, 1966). Warot *et al.* (1991) found that GBE 600 mg significantly improved long term memory compared with placebo but had no effect on critical flicker fusion or choice reaction time.

The efficacious effects of acute doses of GBE on memory and cognitive functioning in asymptomatic volunteers are less well established than the chronic effects of this compound in clinical groups. Future studies in asymptomatic volunteers need to confirm the memory specific activating effect of GBE, identify the specific memory components that are most likely to show efficacy and to determine the optimum dose for such efficacy. The lack of standardization of the GBE extracts used in some past studies has created difficulties in determining a therapeutic dose for cognitive functioning. According to the monograph published by Commission E in Germany the only acceptable extracts for therapeutic use are those with a herb-to-extract ratio in the range of 35:1 to 67:1 (average: 50:1) and with a specific range of flavone glycoside and terpene lactone constituents.

The purpose of this study was to investigate the effects of a range of acute doses of a GBE extract, on a wide range of cognitive and psychomotor variables, in a group of asymptomatic volunteers aged 30–59 years, in order to confirm the memory specific activating effect of GBE, identify the particular memory components that are most likely to show efficacy and to determine the optimum dose for efficacy. Two memory tasks were selected to assess the central executive (immediate word recall) and articulatory loop (Sternberg's Short Term Memory Scanning Task) components of working memory while a third assessed the verbal component of long term memory (delayed word recall). Also, in order to confirm the memory specificity of GBEs therapeutic effect, a number of other measures were included. They consisted of subjective measures of sedation and sleep and objective measures of cognitive and psychomotor activity, including attention and behavioural activity. The GBE used consisted of the standardized *Ginkgo* special extract LI 1370 (Lichtwer Pharma) the composition of which is in accordance with the requirements of the German monograph.

MEASURES AND METHODS

Subjects. Thirty six asymptomatic volunteers (14 females, 22 males) between the ages of 30 and 59 (mean age 43.6 years) participated in the study. Approval was obtained from the University of Surrey Ethics Committee and the subjects gave written informed consent prior to admission to the study. All subjects were in good physical and mental health and free from concomitant medication. Subjects were trained on the experimental measures to a performance plateau to mitigate against learning effects before proceeding to the study.

Design. This was a randomized, double-blind, placebo controlled, 5-way cross-over study. Volunteers received GBE 150 mg (50 mg t.d.s.) and GBE 300 mg (100 mg t.d.s.), GBE 120 mg and 240 mg *mane* and placebo. Each treatment was taken for a period of 2 days and was separated by a 5 day or more washout period. The allocation of subjects to treatments was by a pre-determined randomization schedule, ensuring balanced groups. For each treatment, following baseline measures, the first medication was administered at 0900 h. The second and third medications were administered at 1500 and 2100 h respectively. The test battery was conducted pre-dose (0830 h) and then hourly until 2100 h, when subjects departed. This was repeated on day 2 for each treatment. The test battery consisted of Sternberg's short term memory scanning task (STM), Stroop colour task (SCT), word recall test (immediate recall WRi and delayed recall WRd), critical flicker fusion (CFF), choice reaction time (CRT), digit symbol substitution task (DSST), line analogue rating scales for subjective sedation (LARS), Leeds sleep evaluation questionnaire (LSEQ) and actigraphy (activity monitoring). Subjective sedation and word recall were assessed at frequent, not hourly, intervals post dose and actigraphy assessed motoric activity continuously during the treatment period.

Procedure. Volunteers, fasted overnight, arrived at the study centre at 0730 having been advised to abstain from alcohol, for 24 h before the assessment visit; and caffeinated products, from 2400 h the night before an assessment. All volunteers were breathalysed to ensure compliance with no alcohol protocol; following which actigraphs were attached and worn for the entire study period. They completed a baseline test battery at 0830, including the LSEQ. Further assessments on the battery were made every hour until the final assessment at 2100 h. Subjects received their first medication at 0900 h. The second and third medications were administered at 1500 and 2100 h respectively. There were three short breaks during which volunteers were able to eat a light lunch at 1200 h, a snack at 1500 h and a light evening meal at 1800 h. Volunteers left the unit at 2130 h and arrived the following morning at 0730 h. Before leaving on the final day of the study period, actigraphs were removed.

Assessments

Critical flicker fusion (CFF). The CFF task assesses the integrative capacity of the central nervous system (CNS), and more specifically, the ability to discriminate discrete 'bits' of sensory information (Curran, 1990; Hindmarch, 1975). Subjects are required to discriminate flicker from fusion, and vice versa, in a set of four light emitting diodes arranged in a 1 cm square. The diodes are held in foveal fixation at a distance of 1 m. Individual thresholds are determined by the psychophysical method of limits on four ascending (flicker to fusion) and four descending (fusion to flicker) scales (Woodworth and Schlosberg,

1958). CFF has been shown to be sensitive to a variety of psychoactive compounds (Hindmarch, 1982; Hindmarch *et al.*, 1991a, 1991b; Smith and Misiak, 1976) and to the effects of ageing (Curran *et al.*, 1990). A decrease in the CFF threshold is indicative of a reduction in the overall integrative activity of the CNS (Hindmarch, 1980).

Short term memory (STM). High speed scanning and retrieval from short term memory were assessed using a technique based on a reaction time method (Sternberg, 1966). Subjects memorize a random series of one, three or five digits (the stimulus set) which are presented sequentially at a rate of 1.2 s per digit. One second after the final digit of the stimulus set is presented an auditory warning signal sounds. This is followed by a series of twelve single digit 'probes'. Subjects indicated whether each 'probe' was contained within the original stimulus set or not, by pressing one of two mouse buttons as quickly as possible. The rate of presentation of the 'probes' is determined by the subject's rate of response. Two trials of each stimulus set size was carried out. Response time and accuracy were recorded automatically by the computer. Performance on the STM is sensitive to psychoactive compounds (Subhan and Hindmarch, 1984b) and to the effects of ageing (Anders *et al.*, 1972).

Line analogue rating scale for sedation (LARS). The LARS is employed as a measure of subjective effects of psychoactive drugs. Subjects mark a series of 10 cm line analogue scales, indicating their present feeling with regards to a mid-point, which represents their normal state of mind before treatment began. The mean scores of ratings of 'tiredness', 'drowsiness', and 'alertness', presented among several distracter scales, are taken as a measure of perceived sedation (Hindmarch and Gudgeon, 1980). The higher the score (in millimetres), the less alert and more tired and drowsy the subject feels.

Choice reaction time (CRT). The CRT task (Hindmarch, 1975, 1980) is used as an indicator of sensorimotor performance, assessing the ability to attend and respond to a critical stimulus (Sherwood and Kerr, 1993). Subjects place the index finger of their preferred hand on a central starting button, and are instructed to extinguish one of six equidistant red lights, illuminated at random, by pressing the response button immediately in front of the light as quickly as possible. The mean of fifty consecutive presentations is recorded as a response measure of three components of reaction time: recognition, motor and total reaction time. Recognition reaction time (RRT) is the time it takes for the subject to notice the light, being the time between stimulus onset and the subject lifting their finger from the start button. Motor reaction time (MRT) indexes the movement component of this task, and is the time between the subject lifting their finger from the start button and touching the response button. The total reaction time (TRT) is the sum of RRT and MRT. CRT is sensitive to a variety of psychoactive agents (e.g. Hindmarch, 1980; Hindmarch *et al.*, 1991a) and to the effects of ageing (Frewer and Hindmarch, 1988).

Leeds sleep evaluation questionnaire. The LSEQ assesses the effects of psychoactive compounds on sleep and early morning behaviour (Hindmarch, 1975). The subjects mark a series of 10 cm line analogue scales, indicating the direction and magnitude of any changes in behavioural state they experience following the administration of a drug. More specifically, the LSEQ considers the perceived ease of getting to sleep, the quality of sleep, and any hangover effect the following morning.

Immediate and delayed recall of supraspan word lists. Subjects are given 2 min to learn a list of 20 words. This learning period is immediately followed by 2 min free recall period in which they write down as many of the words that they can remember. A delayed free recall period takes place 30 min later. Word lists across different time points are matched for concreteness, imagery, meaningfulness, frequency (Paivio *et al.*, 1968) and number of syllables. Immediate recall is thought to involve the central executive component of working memory (Baddeley and Hitch, 1974; Baddeley, 1986), whilst delayed recall is a measure of explicit memory.

Digit symbol substitution task (DSST). In the DSST (Weschler Adult Intelligence Scale-Revised, 1981) subjects are presented with two rows of blank squares paired with randomly assigned numbers. They are required to substitute each digit with a different nonsense symbol, the key to which is printed at the top of the sheet. This test has been described both as a measure of simple information processing (Parrott, 1991) and of psychomotor performance (Lezak, 1995). It involves sustained attention and visuomotor coordination.

Stroop task. Stroop tasks are based on the finding that the naming response is slower when a colour word is printed in a different colour ink (incongruent condition) compared to when it is printed in the same colour ink (congruent condition). This 'Stroop effect' has been described as a failure of selective attention in the presence of distracting information (Lezak, 1995; Rusted and Warburton, 1989). Therefore, a decrease in the magnitude of the Stroop effect is indicative of an improvement in selective attention.

Wrist actigraphy. Subjects were required to wear a wrist actigraph (Ambulatory Monitoring Inc. AMA-32 Mini-Motionlogger™, Ardsley, New York) on their non-dominant wrist for the duration of each test period. The actigraph consists of a piezo-electric bimorph weighted cantilevered beam that detects motion in all three axes and generates a signal voltage. The actigraph was set up to record in zero crossing mode (ZCM) with an amplifier setting of 18 and a 10 s recording epoch. In ZCM, each crossing of the reference voltage during an epoch is counted, this gives a measure of the frequency but not the intensity (amplitude) of the movements. Actigraphs measure continuously, indicating the number of zero crossings registered at the end of each consecutive recording epoch. Data were downloaded from the actigraph onto a personal computer. Mean behavioural activity over the whole recording period and sleep efficiency for the intervening night were automatically calculated using the ACTION3 software and its validated sleep/wake algorithm (AMI, Ardsley, New York). It has been shown that a reduced behavioural activity indicated by the actigraphy is reflected in both the psychometric and subjective assessment of sedation, psychomotor

Table 1. Psychometric results by dose, age and day — mean area under curve (standard deviations)

Age (years)	GBE 120 mg		GBE 150 mg		GBE 240 mg		GBE 300 mg		Placebo	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Delayed word recall (no.)										
30–39	−1.19	−1.53	−1.99	−2.47	0.15	−0.90	−1.56	−0.56	−1.15	−0.90
	(1.65)	(2.39)	(1.10)	(1.02)	(1.03)	(0.28)	(1.32)	(1.13)	(2.30)	(2.62)
40–49	−1.60	−1.33	−1.53	−2.02	−1.15	−1.17	−0.91	−0.79	−1.8	−1.50
	(1.26)	(1.47)	(1.32)	(1.63)	(1.99)	(2.20)	(1.44)	(1.49)	(2.15)	(2.04)
50–59	−1.54	−1.37	1.25	−1.34	−2.23	−1.91	−1.71	−1.31	−1.69	−2.12
	(2.07)	(1.90)	(1.84)	(1.98)	(1.69)	(1.24)	(1.28)	(1.37)	(1.43)	(1.60)
Line analogue rating scales for sedation (mm)										
30–39	−6.08	−2.88	−2.84	−5	−1.97	−2.55	−6.66	−4.85	−2.84	1.69
	(11.44)	(6.44)	(12.04)	(13.51)	(8.72)	(2.76)	(10.59)	(10.88)	(10.33)	(6.93)
40–49	2.02	3.21	0.10	1.93	0.12	1.32	1.91	2.31	2.99	4.40
	(3.93)	(7.21)	(8.04)	(8.86)	(5.74)	(7.29)	(3.39)	(5.97)	(2.96)	(2.91)
50–59	0.14	1.78	−1.35	1.27	−0.17	−0.80	2.15	2.48	−3.43	−2.75
	(7.83)	(7.78)	(5.84)	(8.95)	(4.57)	(2.33)	(7.64)	(8.08)	(9.97)	(9.33)
Stroop (ms)										
30–39	13.52	14.30	−10.60	−4.68	−13.56	−3.32	18.56	15.64	−21.15	−26.65
	(55.20)	(55.36)	(39.17)	(31.29)	(53.08)	(56.34)	(42.83)	(44.19)	(35.17)	(69.85)
40–49	25.91	43.19	39.85	30.91	24.04	31.32	6.03	−4.98	32.37	36.81
	(49.51)	(61.63)	(39.93)	(48.23)	(45.21)	(60.23)	(79.07)	(92.71)	(72.68)	(72.08)
50–59	77.19	81.26	38.90	52.56	54.88	50.66	−22.32	3.07	23.30	40.28
	(92.89)	(89.53)	(128.66)	(149.37)	(122.56)	(119.40)	(63.20)	(79.69)	(42.71)	(37.97)
Recognition reaction time (ms)										
30–39	8.16	17.16	21.78	−2.66	17.79	32.42	4.10	19.29	14.64	15.52
	(22.99)	(34.47)	(45.74)	(35.54)	(26.54)	(52.10)	(31.81)	(48.66)	(24.32)	(21.11)
40–49	11.96	13.00	6.65	3.83	8.49	12.11	−4.96	−10.73	9.57	6.82
	(13.37)	(19.71)	(33.38)	(29.38)	(15.60)	(15.76)	(28.83)	(46.50)	(19.43)	(28.22)
50–59	31.07	33.51	21.10	18.84	10.89	−0.04	24.12	25.71	3.93	23.06
	(13.98)	(17.96)	(15.62)	(24.46)	(27.28)	(33.23)	(29.46)	(31.96)	(32.33)	(36.86)
Motor reaction time (ms)										
30–39	−9.17	−15.99	−2.02	−8.01	−3.43	−14.78	−1.07	−1.94	10.82	6.91
	(24.31)	(27.31)	(39.74)	(35.33)	(27.36)	(34.34)	(34.91)	(41.06)	(23.87)	(30.72)
40–49	−5.03	−10.14	6.10	4.70	5.42	7.95	−3.40	−0.03	5.82	0.73
	(13.27)	(22.50)	(17.39)	(22.26)	(15.19)	(29.25)	(21.98)	(23.92)	(15.84)	(27.53)
50–59	0.93	3.01	8.25	8.55	19.95	6.62	−8.95	−4.41	20.67	11.03
	(20.75)	(32.17)	(23.88)	(24.81)	(32.85)	(32.76)	(22.74)	(25.82)	(23.20)	(25.06)
Digit symbol substitution task (no.)										
30–39	−1.98	−4.75	−1.42	−1.20	−1.92	0.85	−1.17	1.21	−2.51	0.72
	(4.65)	(5.03)	(6.46)	(8.66)	(5.31)	(5.70)	(3.23)	(4.70)	(3.96)	(5.83)
40–49	0.99	1.00	−1.47	−2.12	−1.36	−1.25	0.37	0.85	0.88	1.59
	(3.05)	(4.45)	(4.03)	(4.70)	(3.51)	(3.16)	(4.55)	(6.47)	(4.39)	(5.37)
50–59	0.12	−1.56	−1.17	0.92	−1.70	−0.15	−0.63	1.52	−0.72	−1.14
	(4.82)	(6.17)	(6.07)	(6.50)	(4.78)	(6.03)	(5.96)	(7.57)	(4.96)	(5.51)

performance, and arousal (Stanley, 1997; Stanley and Hindmarch, 1997; Stanley *et al.*, 1999).

Statistical analysis. For all variables, except those for the LSEQ and actigraphy, changes from baseline on day 1 were calculated and the area under the resulting response time curve (AUC) was calculated using the truncated trapezoidal rule and normalized by dividing by the duration of the test period. Table 1 presents the means for AUC. Results were analysed using a three-factor repeated measures ANOVA, with age as a between subject factor, and treatment and time within subject. Age had 3 levels, treatment 5 levels (A, B, C, D, E) and time 2 levels (days 1 and 2). Post hoc pairwise comparisons between treatment means were performed using Newman–Keuls tests. Results were available for 31 subjects, although three did not complete day 2 of one cycle. For missing individual data points the results were estimated by calculating the mean of the two on either side of the missing data point for that subject.

RESULTS

Sternberg short term memory scanning

Analysis of variance on the area under the curve showed a marginally significant main effect of treatment ($F(4, 96) = 2.43$, $p = 0.053$) and a significant interactive effect of treatment and day ($F(4, 96) = 3.195$, $p = 0.016$). Post hoc tests for the interactive effects of treatment × day showed that the reaction times for GBE 120 mg and GBE 300 mg were significantly faster than placebo on each day of treatment while GBE 240 mg was significantly

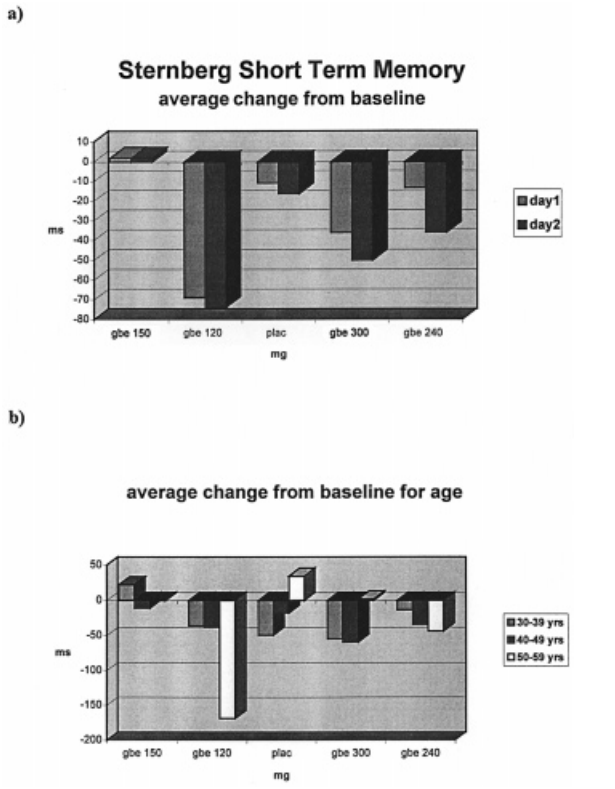

Figure 1. The effects of GBE 150 mg, GBE 120 mg, GBE 300 mg, GBE 240 mg and placebo on reaction time (ms) in the Sternberg short term memory scanning task (STM). Lower scores indicate faster performance. (a) Values are plotted as average differences from baseline (day 1), on days 1 and 2. GBE 120 mg was significantly faster than placebo and all other treatments on each day ($F(4, 96) = 3.195$, $p = 0.016$). (b) Average changes from baseline for each age group.

faster than placebo on day 2 (Fig. 1). The enhancing effect of GBE on performance was most evident for GBE 120 mg. GBE 120 mg led to a mean decrease in reaction time of 69.0 ms on day 1 and 73.8 ms on day 2. Although there were no interactive effects of treatment and age, the effect of GBE 120 mg was most pronounced for the oldest age group; subjects aged 50–59 years showed a decrease in reaction time of 165.6 ms on day 1 and 172.2 ms on day 2.

Immediate and delayed word recall

There were no significant main effects of treatment, age or time, and no significant interactive effects of these factors on the average number of words recalled in either the immediate or delayed recall tests. However, examination of the AUC means indicated that GBE 120 mg and GBE 240 mg increased the overall number of words recalled in the immediate recall test ($F(4, 100) = 1.194$, $p = 0.318$) (Fig. 2). This increase was more pronounced for GBE 120 mg. Whereas the overall increase for GBE 240 mg was caused by an improvement in performance on the first day of treatment only, the improved performance for those receiving GBE 120 mg on the first treatment day was actually enhanced on the second day. This is in accordance with the finding of improved performance on the Sternberg memory scanning task for GBE 120 mg.

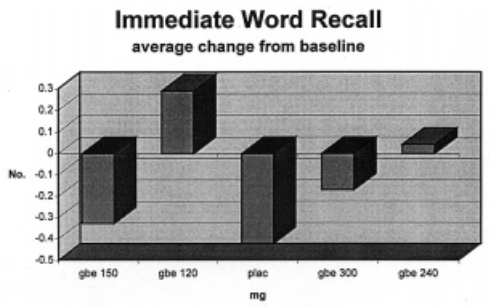

Figure 2. The effects of GBE 150 mg, GBE 120 mg, GBE 300 mg, GBE 240 mg and placebo on immediate word recall. Higher scores represent an increase in the number of words recalled. Values are plotted as average differences from baseline (day 1). GBE 120 mg and GBE 240 mg were associated with an increased number of words recalled ($F(4, 100) = 1.194$, $p = 0.318$).

Critical flicker fusion

There was a significant main effect of treatment on CFF threshold ($F(4, 92) = 2.572$, $p = 0.043$). The mean CFF threshold for GBE 120 mg were higher than placebo and all other treatments (Fig. 3). However, post hoc analysis showed that there was a significant overall difference only between GBE 120 mg and GBE 240 mg. Compared with the baseline, GBE 120 mg led to a mean increase of 0.04 Hz while GBE 240 mg decreased CFF threshold by 0.66 Hz. The average increase in CFF threshold for GBE 120 mg was the greatest in the oldest age group; subjects aged 50–59 years had an increase of 0.46 Hz. The age related effect of GBE 120 mg on CFF is consistent with the finding of improved performance in the Sternberg short term memory scanning task for the 50–59 year age group.

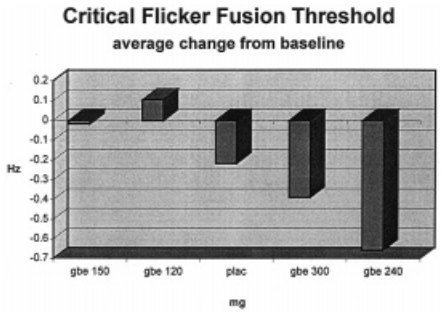

Figure 3. The effects of GBE 150 mg, GBE 120 mg, GBE 300 mg, GBE 240 mg and placebo on critical flicker fusion threshold (CFFT). Higher scores in hertz (Hz) indicate improved performance. Values are plotted as average differences from baseline (day 1). GBE 120 mg had higher CFF thresholds than placebo and all other treatments, and was significantly higher than GBE 240 mg ($F(4, 92) = 2.572$, $p = 0.043$).

Choice reaction time

There were no significant main effects of treatment, time or age and no significant interactive effects between any of these factors on total reaction time (TRT) or on motor reaction time (MRT). Treatment, age and time had a significant interactive effect on recognition reaction time

(RRT) ($F(98, 92) = 2.225$, $p = 0.0323$). When the treatment × age results interaction was examined separately for each day no significant differences in reaction time emerged. However, examination of the means for AUC indicated that reaction time was fastest on the first day for all treatments except GBE 150 mg, that GBE 300 mg was associated with the most rapid reaction time and that those aged 40–49 years maintained the fastest reaction time. Although no significant treatment effects emerged for MRT, the pattern of response was consistent with that for CFF, STM and Wri in that the average change from baseline was faster for GBE 120 mg than placebo and all other treatments on each day.

Stroop colour task

When the AUC for the difference between the time for matched and unmatched colours was analysed, there was only a significant effect of age ($F(2, 23) = 3.649$, $p = 0.042$). Post hoc tests showed a significant difference between the 30–39 age group and those between 50 and 59 years. Compared with the baseline, the mean reaction time of the younger age group decreased by 1.8 ms while that of the older age group increased by 40 ms.

Digit symbol substitution task

Analysis of area under the curve for the number of symbols completed showed a significant effect of time ($F(1, 25) = 14.248$, $p = 0.009$), and significant interactive effects of treatment × time ($F(4, 100) = 4.505$, $p = 0.002$) and treatment × time × age ($F(8, 100) = 2.059$, $p = 0.047$). All treatments, with the exception of GBE 300 mg, were associated with a drop in the number of symbols completed on day 1 while GBE 100 × 3 mg was the only treatment which increased the mean number of symbols on day 2. Compared with the baseline, GBE 100 × 3 mg led to a mean increase of 1.15 symbols on day 2. When pairwise comparisons were performed for day 2, GBE 300 mg was not significantly different to placebo but was significantly different to GBE 150 mg and GBE 120 mg.

Line analogue rating scale for sedation

GBE had no significant effects on perceived arousal but there was a significant effect of time ($F(1, 24) = 6.192$ $p = 0.02$), with subjects experiencing lower arousal on day 2. Compared with the baseline, all groups had increases in levels of alertness on day 1 with the exception of placebo and GBE 240 mg. GBE 120 mg was associated with the greatest increase from baseline. However, all groups with the exception of GBE 300 mg and GBE 240 mg had decreases in perceived arousal on day 2. Perceived arousal for those receiving placebo was lowest on day 2; compared with the baseline there was an average increase in score of 3.02 mm.

Leeds sleep evaluation questionnaire

There were no significant effects ($p > 0.05$) of treatment or age, and no significant interactive effects of these factors on getting to sleep (GTS), quality of sleep (QOS) or awakening from sleep (AFS).

Actigraphy

There were no significant main effects of treatment for levels of behavioural activity. There was also no difference between treatments in the sleep efficiency in the intervening night when the volunteers slept at home.

DISCUSSION

The purpose of this study was to look at the effects of different dose regimens of *Ginkgo biloba* extract on a test battery assessing memory and other aspects of cognitive and psychomotor functioning, in order to confirm the memory specific enhancing effects of GBE, identify the particular memory components that are most likely to show efficacy and to determine the optimum dose for such efficacy. A secondary objective was to examine the role of age as a mediator of the effects of GBE.

The results show that the effects of GBE extract on aspects of cognition in normal healthy volunteers are more pronounced for memory, particularly working memory, than for arousal or selective attention, that these effects may be dose dependent though not in a linear dose related manner and that GBE 120 mg produces the most evident effects of the doses examined. Additionally, the results suggest that the memory enhancing effects of GBE in asymptomatic volunteers are more likely to be apparent in individuals aged 50–59 years.

When the area under the curve was examined, reaction times in the Sternberg short term memory scanning task for all GBE treatments, except GBE 150 mg, were significantly faster than placebo on day 2 of treatment while the reaction times for GBE 120 mg and GBE 300 mg were significantly faster than placebo on both days of treatment. This effect on STM was most pronounced for GBE 120 mg and was more evident in the oldest age group. Subjects aged between 50 and 59 years and receiving GBE 120 mg showed an overall decrease in reaction time of 119.7 ms. Furthermore, consistent with the finding of improved performance on the Sternberg memory scanning task for GBE 120 mg, GBE 120 mg was the only treatment which increased the average number of words recalled from baseline in the immediate recall test on the second day of treatment. However, this effect was not statistically significant.

Although there was an overall treatment effect for critical flicker fusion threshold, none of the GBE doses was significantly different to placebo. However, GBE 120 mg was associated with a higher CFF threshold than placebo and all other treatments. Interestingly, this study did not demonstrate a significant effect of GBE on either Stroop or the recognition component of the choice reaction time task, measures of selective attention, or on the digit symbol substitution task, a measure of sustained attention. Although some studies have shown that GBE can enhance attention in long duration vigilance type tasks (e.g. Pidoux, 1988), most studies which have indicated improvements in vigilance have used pharmaco-electroencephalography (EEG) rather than psycho-

metric tests. The DSST used in the present study assesses a number of cognitive processes simultaneously, including sustained attention, short term memory and psychomotor speed; however its selectivity for sustained attention may not be sufficiently sensitive. Furthermore, while the DSST was completed during a 90 s time period sustained attention and vigilance tasks are normally performed over at least a 10 min period.

The results of this study are almost completely in accordance with the results of Subhan and Hindmarch's (1984a) study in which they found that GBE 600 mg did not affect critical flicker fusion, choice reaction time or subjective ratings of arousal, but did improve performance in the Sternberg memory scanning task. The present study also indicates that the effects of GBE are more evident for memory, particularly working memory as assessed by the Sternberg task. Although there were no significant effects of GBE on any other variables, there is some evidence to suggest that GBE may have an activating effect on CNS arousal as indicated by increases in CFF threshold for GBE 120 mg. However, in contrast to the findings of Subhan and Hindmarch, these results suggest that the optimum dose for cognitive enhancement may be significantly lower than that found in their study, as well as other past investigations. This is possibly related to the lack of standardization of the GBE extracts used in past studies.

The majority of studies which have shown effects of GBE have used elderly subjects who are already showing evidence of cognitive impairment. These studies have generally indicated that the positive effects of GBE are not observed until at least 4–6 weeks of treatment (e.g. Kleijnen and Knipschild, 1992). However, a number of studies have shown positive effects of GBE on cognitive functioning in asymptomatic volunteers. Although the results of these studies have not been definitive, the most consistent finding from these studies is that GBE primarily enhances memory (Warot *et al.*, 1991). Most of these studies have involved the administration of a single, usually large, GBE dose (e.g. Allain *et al.*, 1993).

Identifying the enhancing effects of GBE in normal asymptomatic volunteers may be especially problematic. Difficulty in the detection of positive effects with cognitive enhancers in asymptomatic volunteers may have parallels with stimulant drugs (Fagan *et al.* 1988). This is because improved performance may be more difficult to detect than sedation, because normal subjects under normal conditions are working close to their optimum performance, and therefore have less room for improvement than for impairment, and also because cognitive enhancers would appear to be less global in their effects than sedative drugs. All of these issues highlight the need for a wide ranging sensitive and reliable test model which will focus on various individual components of information processing, particularly those for memory, as was used in the present study. This model would also be of value in assessing the effects of chronic doses over at least a similar time period to that examined in clinical groups.

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